STUDIES ON POTATO PROTEINS

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Abstract

Protein patterns of four potato tuber varieties grown in the Northeastern United States were compared by electrophoresis and electrofocusing and found to be distinctly different. Molecular sizes of protein subunits of all four varieties were found to be uniform when charge differences between proteins were masked and disulfide bonds ruptured. Preliminary fractionation of Katahdin variety tuber proteins by dialysis against water yielded 25% globulin and 75% albumin. Further fractionation of the acidic proteins of the globulin fraction by density gradient isoelectric focusing at pH 4-6 separated three fractions isoelectric at pH 4.2, 4.4, and 5.3. Amino acid compositions of the three fractions were similar.

Resumen

Modelos de proteinas de 4 variedades de tubérculo de papa cultivadas en los estados del Noroeste fueron comparados por electroforesis y electroenfoque encontrándose que eran claramente diferentes. Los tamaños moleculares de las sub-unidades protéicas, de las cuatro variedades se encontraron uniformes cuando las diferencias de cargas entre las proteinas fueron encubiertas y los enlaces disulfitos rotos. Fraccionamiento preliminar de las proteinas de tubérculos de la variedad Katahdin mediante diálisis, con agua, rindió 25% de globulina y 75% de albúmina. Un mayor fraccionamiento de proteínas acídicas de la fracción globulina por gradientes de densidad con enfoque isoeléctrico a pH 4-6 separaron 3 fracciones isoeléctricas a pH 4.2, 4.4 y 5.3. La composición de aminoácidos de las tres fracciones fueron similares.

Introduction

Bioassays have verified the high protein quality of potatoes (9); however, the possibility exists of improving this quality through breeding and selection of potatoes (7, 12, 15). A prerequisite for realizing this potential is

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knowledge of the structure and function of singular protein components and factors affecting them whether of genetic, physiological, pathological, or environmental origin.

Important contributions have been made in recent years. Lindner *et al.* (11) fractionated potato proteins according to traditional protein solubility classes, and Stegemann and Loeschcke (17), Desborough and Peloquin (6), and Nagasone *et al.* (13) demonstrated many additional fractions by electrophoresis and chromatography. Using electrophoretic techniques, Stegemann *et al* (18) studied properties and changes in tuber proteins of several European varieties as a consequence of aging. Richardson (14) reported the amino acid sequence of a subunit of one of the chymotryptic inhibitors found in potato tubers.

This report discloses progress in the investigation of chemical and physical properties of proteins of tuber varieties grown in the Northeastern United States.

Materials and Methods

Potato tubers (Katahdin, Kennebec, Merrimack, and Wauseon vars.) were obtained from Aroostook Farms, Presque Isle, Maine, USA, freshly harvested September 1976, and stored at 10°C. For sap (juice) preparation, various sizes of tubers were washed, peeled, and small pieces were homogenized for 2 minutes in 0.1M phosphate (pH 6.8) containing 0.1% $NaHSO_3$ and 0.1% diethyldithiocarbamate (DIECA). The slurry was strained through four layers of cheese cloth, and the filtrate was mixed with a few drops of toluene, refrigerated overnight, and then clarified by centrifugation (15,000×g). This clarified sap was divided into smaller samples and stored frozen until use.

Protein concentration was rapidly estimated according to the method of Bradford (3), with bovine gamma globulin used as protein standard.

Proteins were concentrated by use of a stirred ultrafiltration cell (Amicon Corp., Lexington, Mass.) with membranes designed to retain macromolecules of mol. wt. 10,000 and larger. Later in the work, they were concentrated more rapidly by a method developed by Allington *et al.* (1) involving a combination of electrophoresis and filtration.

Analytical electrophoretic protein separations were carried out in horizontal gels 2 mm thick, 5.1% acrylamide/2.6% bisacrylamide, buffered with 0.1 M Tris-borate, pH 8.9, on a commercially available instrument (LKB 2117 Multiphor, LKB-Produkter AB, Bromma, Sweden), and also in gel rods. Separation of protein subunits and estimation of molecular weight were accomplished by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (16, 21). Isoelectric focusing in polyacrylamide gel rods also was used for analytical protein separations, as were thin layer gels according to the technique of Awdeh *et al.* (2) as modified by Vesterberg

(20). Vesterberg (19) detected protein in the gels by staining with Coomassie Blue R-250.

Larger amounts of protein, up to 20 mg, were separated by density gradient isoelectric focusing in an LKB 8100 column (volume 110 ml) according to the manufacturer's instructions. Wide range (pH 3-10), as well as narrow range, pH gradients used in the process were produced with commercially available carrier ampholytes. Focused protein fractions collected from the column were stripped of carrier ampholytes by gel filtration on a 1.5×75 cm column of LKB Ultrogel AcA 22. The protein fractions were eluted in the gel void volume.

Amino acid composition of acid-hydrolyzed protein fractions was determined with a Beckman Model 119B automatic amino acid analyzer. Tryptophan was not estimated.

Results and Discussion

Comparison of Varieties by Polyacrylamide Gel Electrophoretic Techniques

The electrophoretic patterns of the soluble proteins of the four varieties of tubers are shown in Fig. 1. Electrophoresis at pH 8.9 resulted in anodic migration of most proteins of all four varieties; only one or two protein zones, apparently common to all four varieties, were retrograde. While electropherograms of proteins are finding increased usage in genetic and taxonomic identification studies, the retrograde proteins shown in Fig. 1 would not have been disclosed by the more commonly used vertical gel rod or slab techniques.

Figure 2 shows the tuber proteins of the four varieties after horizontal electrofocusing in thin-layer gel, pH range 3.5-10. Even though the patterns exhibit an obvious familial relationship under the indicated experimental conditions, close inspection reveals that each pattern is different from every other. This is more evident if the proteins are electrofocused at different pH ranges to spread the pattern, especially the acidic proteins.

The molecular weight distribution of tuber protein subunits was investigated by gel electrophoresis in buffer containing sodium dodecyl sulfate. Disulfide bonds were cleaved with dithiothreitol (DTT) (5), and the reduced subunits, or protein fragments, were complexed with SDS. The subunits then migrated in the gel at rates proportional to their sizes (16). The polypeptides of the four varieties examined were resolved into two groups with estimated molecular weights of 16,500 and 29,000 on the basis of comparison with the mobilities of calibration proteins, as shown in Fig. 3. The similarities in size distribution of the polypeptides of these varieties suggest the possibility of a common parent protein. Apparently, the differences observed in Figs. 1 and 2 are associated with divergent evolutionary

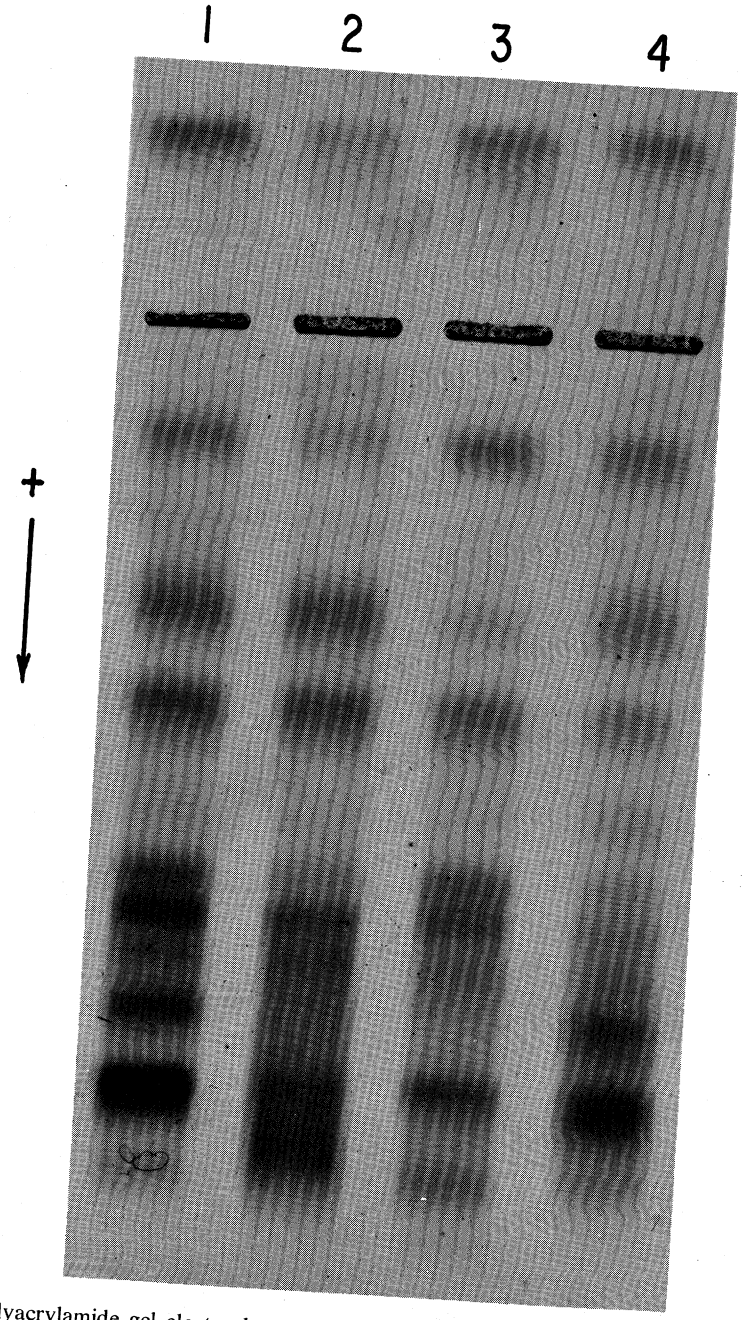

FIG. 1. Polyacrylamide gel electrophoresis of proteins extracted from four potato tuber varieties: (1) Katahdin, (2) Kennebec, (3) Merrimack, (4) Wauseon. Gel: 5.1% acrylamide/2.6% bisacrylamide, Tris-borate buffer, pH 8.9.

developments in charge distribution. These results and conclusions are in general agreement with the findings of Stegemann *et al.* (18).

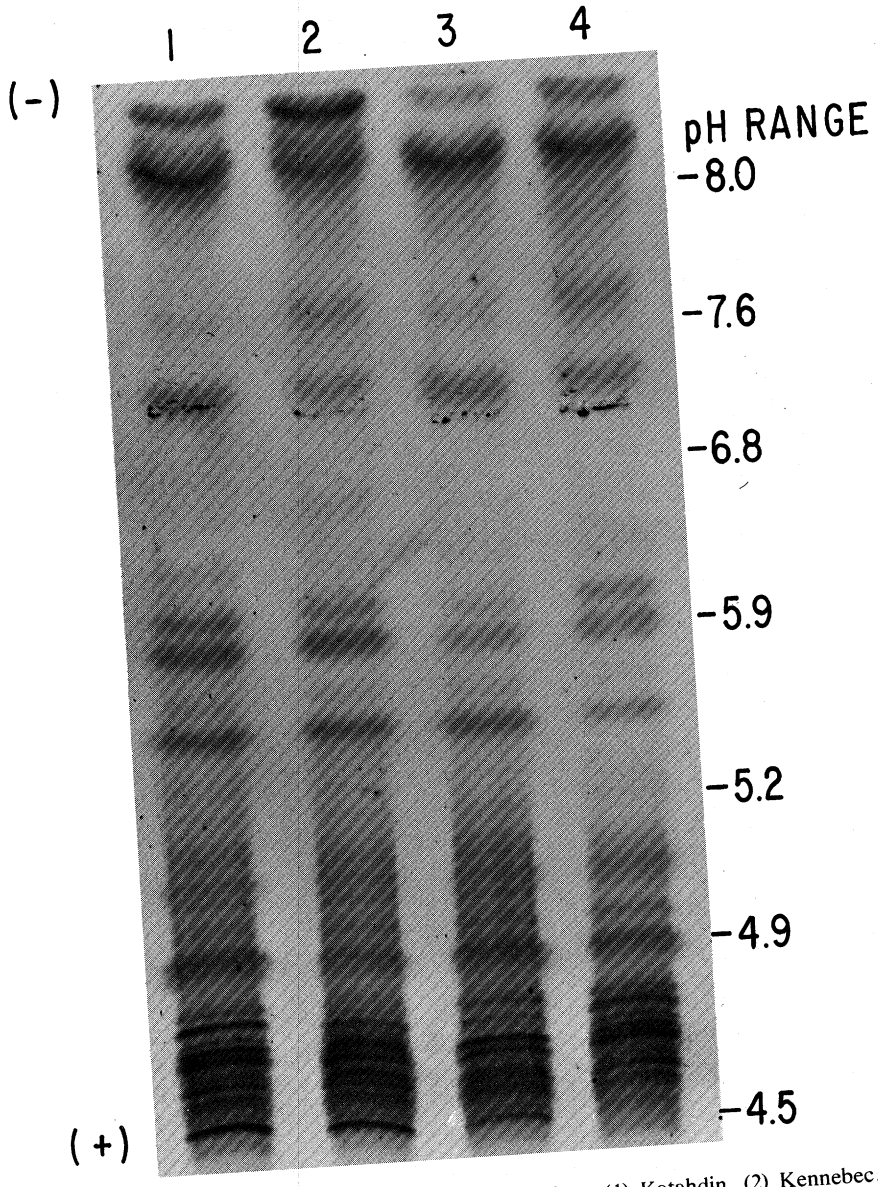

FIG. 2. Isoelectric focusing of proteins extracted from (1) Katahdin, (2) Kennebec, (3) Merrimack, and (4) Wauseon potato varieties on thin layer polyacrylamide gel, pH 3 to 10. Running time was 84 min at 25 W constant power, 4°C. Samples were applied on filter paper strips close to the cathode end of the gel.

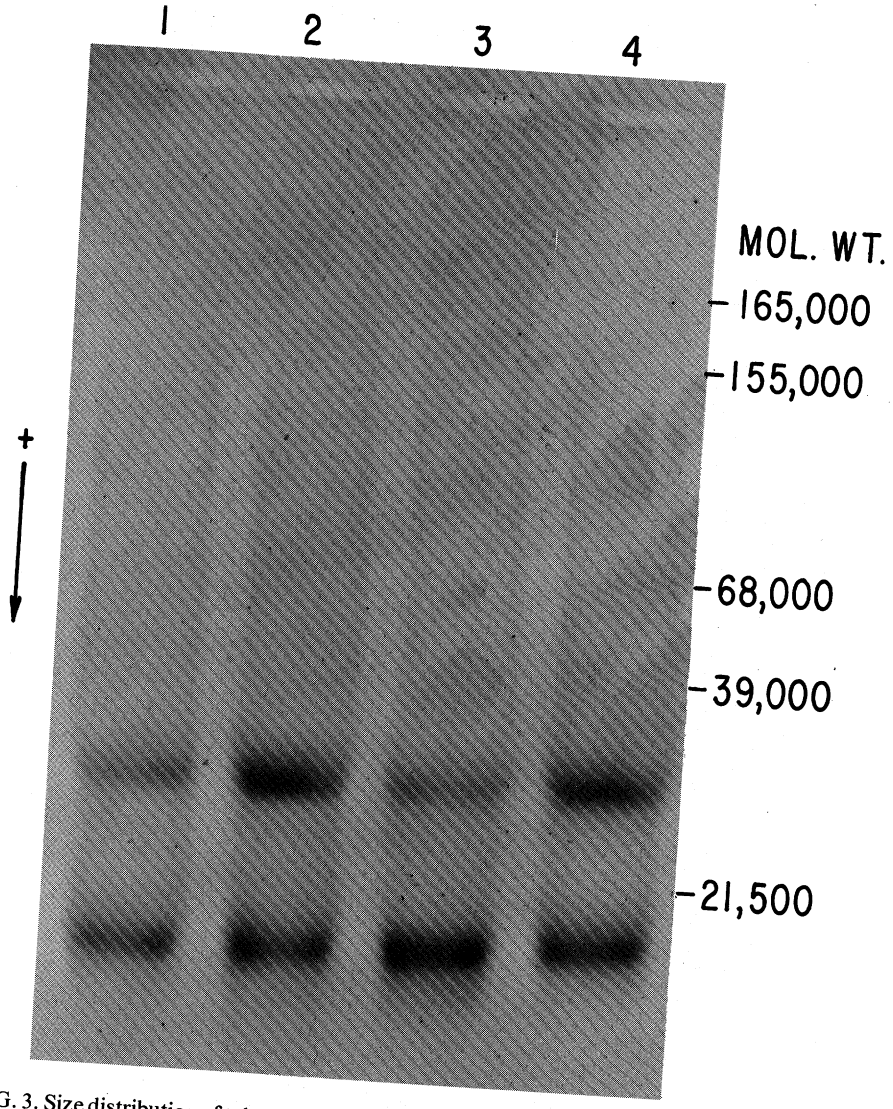

FIG. 3. Size distribution of tuber proteins from (1) Katahdin, (2) Kennebec, (3) Merrimack, (4) Wauseon varieties. SDS/DTT-treated samples in 5% polyacrylamide gel, 0.05 M imidazole buffer containing 0.1% SDS, pH 7.0. Mol. Wt. markers include soybean tryspin inhibitor (21,500), bovine serum albumin (68,000), RNA-polymerase subunits α (39,000), β (155,000), and β′ (165,000).

Protein Isolation and Preliminary Fractionation

The scheme found most useful for preliminary isolation of soluble tuber protein is sketched in Fig. 4. While it is common practice to add reducing agents such as bisulfite during extraction to prevent oxidation of

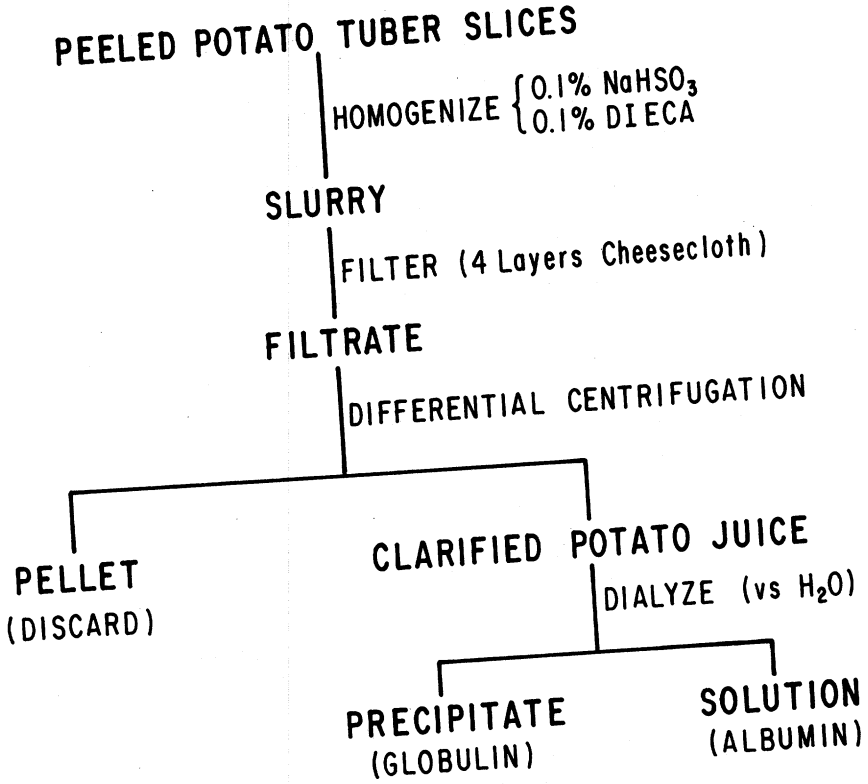

FIG. 4. Preliminary fractionation of potato proteins.

phenolics, they were only partly effective here. However, when used in conjunction with copper chelating reagents such as DIECA (4), the protein extracts could be stored frozen for months without appreciable tanning taking place.

Preliminary fractionation of tuber proteins by exhaustively dialyzing potato juice against water resulted in a water-insoluble fraction, arbitrarily designated as albumin, and a water-insoluble fraction which subsequently proved to be soluble in 5% K_2SO_4. The water-insoluble fraction, or globulin, comprised about 25% of the total potato juice protein. On the other hand, Levitt (10) obtained nearly equal amounts of globulin and albumin by a similar dialysis procedure, whereas Kapoor *et al.* (8) found 46-48% albumin and 26-30% globulin. Although useful for preliminary fractionation, this procedure and others like it should not be assumed to give sharp distinction between solubility classes (such as globulin and albumin). The complexity and extreme instability of potato tuber proteins tend to make

distinctions based on solubility unreliable. Therefore, designation of these protein fractions as globulin and albumin here is strictly for convenience.

Isoelectric Focusing in Density Gradient

Among the several methods tested for further fractionation, electrofocusing in a density gradient offered the most promise. Fig. 5 shows a typical separation of globulin (A) and albumin (B) fractions by electrofocusing within a pH range of 3.5-10, stabilized by a linear glycerol

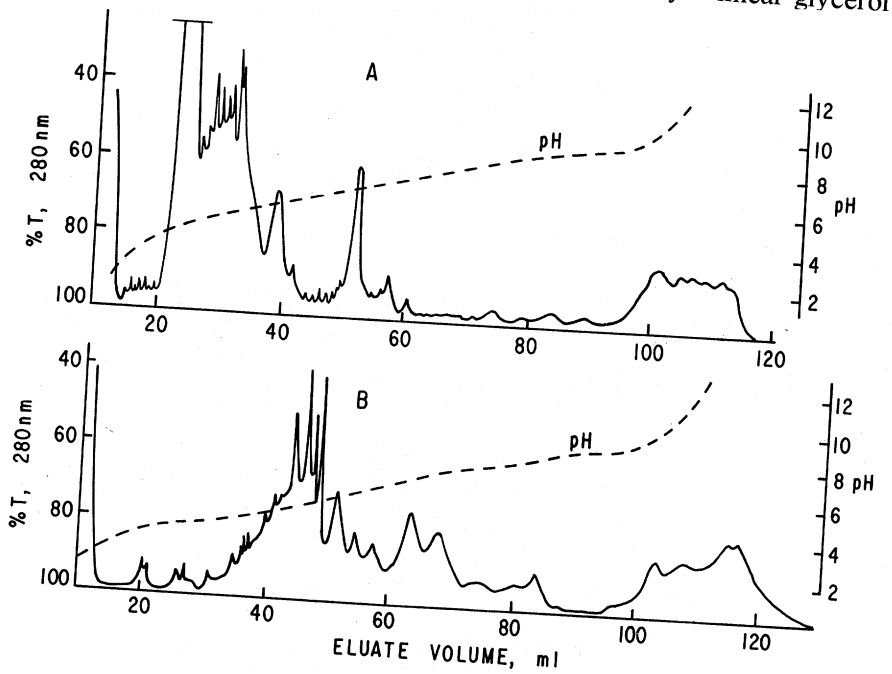

FIG. 5. Preparative isoelectric focusing of globulin (A) and albumin (B) fractions from Katahdin var. tuber proteins. 20 mg each sample focused in 1% (w/v) Ampholine, pH range 3.5-10, in a 0-60% (w/v) glycerol density gradient. Focusing time: 10 h at 1000 V (final voltage), column temperature 4°C, pH of fractions measured at 10°C.

density gradient (0-60% w/v). Most of the tuber proteins of Katahdin and related varieties are acidic in nature. In both the globulin and albumin fractions, most of the components have isoelectric points between pH 4 and 5.2. Because of the importance of the acidic proteins to the total protein complement of these tuber varieties, representative proteins were isolated from this group by electrofocusing in narrower pH gradients. Fig. 6 is a replica of the preparative fractionation of the globulin fraction electrofocused between pH 4 and 6. The three fractions having apparent isoelectric points of 4.2, 4.4, and 5.3 are clearly resolved.

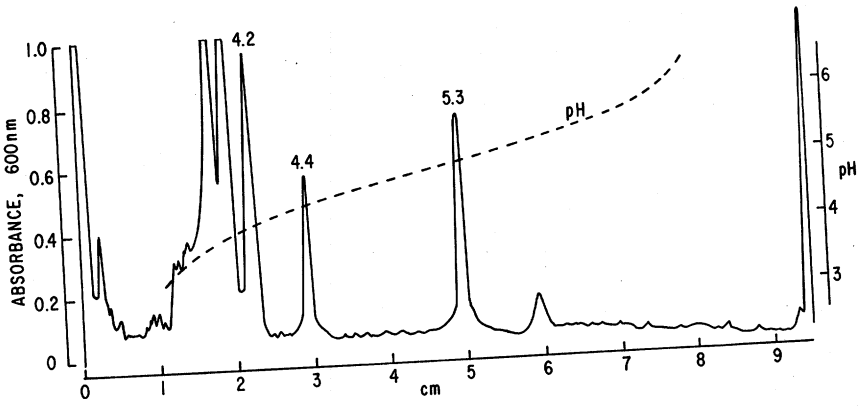

FIG. 6. Densitometric scan of a gel rod stained with Coomassie Blue G-250 to show separation of proteins isoelectric at pH 4.2, 4.4, and 5.3 from the globulin fraction of Katahdin var. tuber proteins. pH gradient 4-6.

Amino Acid Composition of Separated Fractions

The amino acid composition of the three fractions examined thus far (Table 1) is similar. The greater quantities of aspartic and glutamic acid observed would be expected on the basis of the acidic isoelectric points.

TABLE 1. — *Amino acid composition of globulin fractions separated by isoelectric focusing (mol/100 mol).*

Amino acid	pH 5.3	pH 4.4	pH 4.2
Alanine	10.5	8.2	8.2
Aspartic acid	11.9	13.0	10.6
Glutamic acid	9.7	11.8	10.7
Glycine	8.4	10.2	7.2
Proline	5.1	1.7	4.4
Serine	5.6	7.4	4.2
Ornithine	-	-	-
Methionine	-	1.1	1.8
Threonine	7.6	3.9	5.9
Valine	7.6	6.4	6.8
Lysine	6.0	6.5	7.0
Isoleucine	5.8	5.5	6.1
Leucine	11.8	9.6	10.6
Phenylalanine	5.3	4.6	5.2
Histidine	2.3	2.2	2.0
Arginine	2.1	4.2	3.8
½ Cystine	-	-	-
Tyrosine	-	3.3	5.6
Tryptophan	N.A.[1]	N.A.[1]	N.A.[1]

[1]Not analyzed

The amino acid content of other tuber proteins not so closely related isoelectrically may be found to differ substantially.

Further study of potato proteins with the tools now available should shed more light on their structure and function.

Acknowledgments

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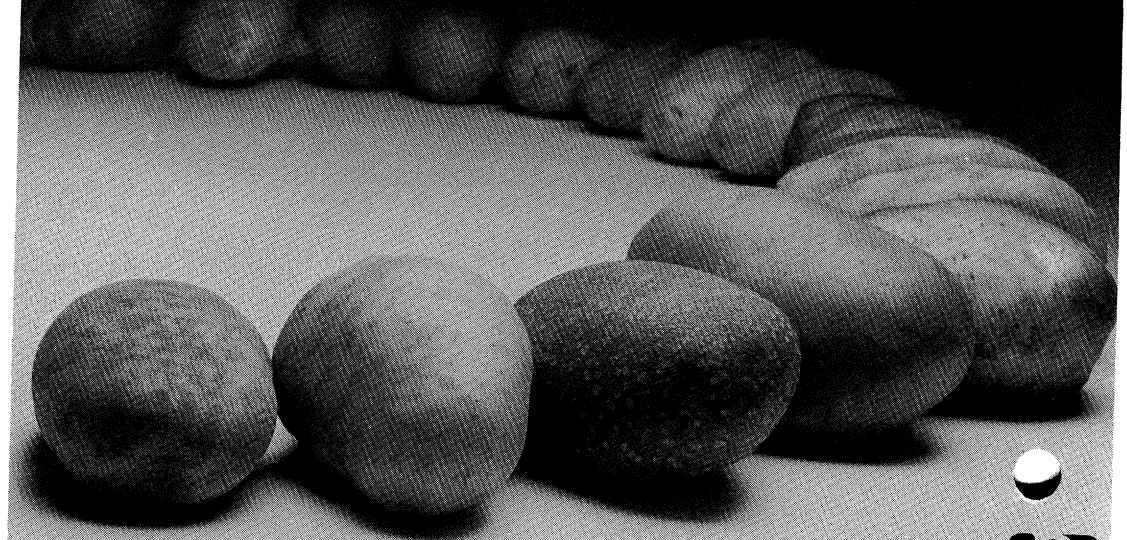

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